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Epidermal Uptake of Nutrients in an Unusual Turbellarian Parasitic in the Starfish *Coscinasterias calamaria* in Tasmanian Waters

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Abstract. The parasitic turbellarian *Acholades asteris* (Neorhabdocoela: Acholadidae) lives encysted on the ambulacral tube-feet of the starfish *Coscinasterias calamaria* in Tasmanian waters. Cysts, each containing one worm, generally occur singly as spindle-shaped protrusions from near the bases of the tube-feet; the cyst wall consists of tube-foot epidermis, with nervous tissue, and part of the outer layer of the tube-foot's two-layered connective tissue sheath. The inner layer, musculature, and coelomic epithelium are not involved, and the tube-foot remains functional. The turbellarian lies within the split outer layer with its anterior towards the base of the tube-foot. It lacks mouth, pharynx, and intestine and feeds via its ciliated epidermis on the surrounding collagenous and cellular materials. These are partially digested by enzymes from epidermal and subepidermal glands, products are pinocytosed, and digestion is completed in epidermal phagosomes formed by fusion of pinocytotic vesicles. Food uptake occurs over the entire body surface but especially anteriorly where the epidermis is thicker, deeply invaginated, and underlain by concentrations of gland cells. In approximately 25% of flatworms examined, the epidermis and adjacent parenchyma contained trophozoites and other stages of *Monocystella* sp., an aseptate gregarine whose other, known, species occur in turbellarian alimentary systems.

Introduction

The Turbellaria are typically free-living flatworms but many foreshadow the exclusively parasitic life styles of the Monogenea, Digenea, and Cestoda by living in various types of permanent symbiotic associations with other animals (Jennings, 1971; Cannon, 1986). The nutritional adaptations of many such symbiotic species are now well known and have been reviewed by Jennings (1977, 1988) and Shinn (1981). Feeding patterns include epi- and entozoic predation, ecto- and entocommensalism, entoparasitic utilization of host tissues or metabolites, and various combinations of these. Patterns of digestive physiology are correlated with feeding habits and range from examples identical with those of free-living predators to instances of partial or total dependence on digestive enzymes obtained from the host. However, even in these latter cases the alimentary system usually remains anatomically well developed, apart from the loss of intrinsic gland cells, and in the majority of symbiotic turbellarians it is the principal, if not the only, route along which nutrients enter the body. Epidermal uptake as a supplementary mode of nutrition has not been demonstrated in such species, unlike the comparable situation in digenetic trematodes (Smyth and Halton, 1983). There are no obvious adaptations for absorption or membrane ("contact") digestion; ultrastructural studies of the epidermis in, for example, the umagillid *Syndesmis franciscana* by Holt and Mettrick (1975) and the graffillids *Paravortex cardii* and *P. karlingi* by MacKinnon *et al.* (1981), have not revealed significant differences in the numbers or sizes of the microvilli occurring between the epidermal cilia, or in the occurrence of epidermal folds or invaginations, when these species are compared with a representative range of free-living turbellarians (reviewed by Tyler, 1984). The possibility of supplementary epidermal uptake cannot be dismissed, as Jondelius (1986) reports the occurrence of coated vesicles and similar structures, suggestive of pinocytosis, in

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the distal regions of epidermal cells in the umagillid *Anoplodium stichopi*.

A minority of entoparasitic turbellarians, notably the Fecampiidae and Acholadidae, have lost all traces of an alimentary system from their adult stages. In these instances epidermal uptake of the nutrients necessary for growth and reproduction would seem to be the only possible mechanism. In the Fecampiidae, species of *Fecampia* and *Kronborgia* live in the hemocoel of various crustaceans (Caullery and Mesnil, 1903; Christensen, 1981; Bellon-Humbert, 1983; Shinn and Christensen, 1985), a habitat in which they are bathed in soluble potential nutrients. In *Kronborgia amphipodicola*, the only species for which relevant data are available, the epidermal microvilli are close-set, clavate, and very much longer (up to 3 μm in height) than those of other turbellarians. These features were interpreted by Bresciani and Køie (1970) as adaptations increasing epidermal surface area for either passive or active absorption. Bresciani and Køie suggest that pinocytosis may also occur but found very few apparently pinocytotic vesicles in the epidermal cells.

The fecampiid *Glanduloderma myzostomatis* lives in the mesenchyme of myzostomid annelids (Jägersten, 1942), while the only known member of the Acholadidae, *Acholades asteris*, occurs in the connective tissue of the tube-feet of a starfish (Hickman and Olsen, 1955). In these habitats they obviously have smaller quantities of soluble nutrients available for epidermal uptake than do *Fecampia* or *Kronborgia* spp., and the absence of orthodox alimentary systems precludes direct ingestion of host tissues in the manner seen in tissue-feeding flatworms such as graffillid and pterastericolid rhabdocoels (Jennings and Phillips, 1978; Jennings and Cannon, 1985; Kent and Olson, 1986) and some digenetic trematodes (Smyth and Halton, 1983).

It is clear from this brief survey that much remains to be learned about the nutrition of those entosymbiotic turbellarians that lack recognizable alimentary systems. Therefore, in the present study the tissue-dwelling rhabdocoel *Acholades asteris* has been examined by histological and histochemical methods, and ultrastructurally so far as was possible with the available material, to ascertain the nature of its food and methods of digestion and assimilation.

Materials and Methods

Specimens of the starfish *Coscinasterias calamaria* (Gray) (Asteroidea: Asteriidae) were collected in November 1986 by SCUBA divers from depths of 8–12 m in D'Entrecasteaux Channel off Tinderbox, S.E. Tasmania. Ambulacral tube-feet bearing yellow to orange cysts containing the turbellarian *Acholades asteris* Hickman and Olsen (Neorhabdocoela: Acholadidae) were removed within 6 h of collection and fixed *in toto* in Bouin's fluid, 90% ethanol or 10% formalin buffered to pH 7.0 with 0.1 *M* sodium phosphate and used at 4°C.

The structure and inter-relationships of the tube-foot, cyst wall, and the turbellarian's body wall and associated glands were studied in paraffin wax serial sections, 4 or 8 μm thick, prepared by standard procedures after Bouin or ethanol fixation. The sections were stained by an alcian blue, periodic acid-Schiff (PAS) and orange G trichrome method for glycoproteins and mucosubstances (Pearse, 1972), Curtis's Ponceau S method for collagen, Ehrlich's haematoxylin and eosin, Feulgen's method for DNA, Giemsa's stain, Gomori's aldehyde-fuchsin method for elastin, Heidenhain's iron haematoxylin and metanil yellow, Mallory's trichrome stain, or the PAS method, with amylase controls, for glycogen.

Endopeptidases produced by the turbellarian were detected after fixation in cold neutral formalin, using the indoxyl acetate method for non-specific esterases (Holt, 1958). Tube-feet and attached cysts were incubated in the standard medium, and in media containing specific activators and inhibitors as described by Hassall and Jennings (1975) and Jennings (1985), for 6 h at 20°C and at pH 4.5, 7.0, or 8.5. Specimens were then washed in 0.1 *M* phosphate buffer, dehydrated in graded ethanols, and serially sectioned in paraffin wax at 4 μm.

All sectioning and subsequent procedures were carried out in England after collection of specimens and preliminary processing in Tasmania. Due to unavoidable circumstances, no appropriately fixed material was available for ultrastructural study, other than tube-feet and cysts fixed and held in 10% neutral formalin. Trimmed portions of these were washed in 0.1 *M* phosphate buffer at pH 7.0, post-fixed for 3 h in 3% glutaraldehyde in buffer, washed in buffer, and treated for 1 h with buffered 2% osmium tetroxide. They were then embedded in araldite and sectioned; thin sections were mounted on formvar-carbon films carried on copper slot grids, stained with uranyl acetate and lead citrate, and examined in a JEOL 1200 EX transmission electron microscope. Other sections, 1–2 μm thick, were stained with Azur II and examined using the light microscope.

Results

Cysts containing *Acholades asteris* were found on 11 of the 14 *Coscinasterias calamaria* examined. In these almost all ambulacra bore infected tube-feet and further incidence data were not recorded. The bright yellow or orange cysts usually occur singly as spindle- or pear-shaped protrusions, 4–5 mm in length when mature, at-

tached near the bases of the tube-feet (Fig. 1A). Cysts were not found beyond the mid-regions of the podial columns, nor on the sucker-discs. Occasionally, two or even three cysts may occur on the same tube-foot; this is most likely in the proximal rather than distal portions of the ambulacrum. Infected tube-feet remain fully functional and show the same amounts of extension, retraction, bending, and adhesion as uninfected ones. The cysts show only limited movements, mainly as changes in length and occasional lateral bending; these are caused by movements of the contained flatworm whose shape within the cyst can be seen when viewed against bright transmitted light.

Histological structure of the tube-foot wall and cyst wall

The histological structure of the ambulacral tube-feet in *C. calamaria* (Figs. 1A, B) does not differ significantly from that described for other asteroids. Briefly, the tube-foot wall consists of a thin cuticle overlaying tall columnar epidermal cells interspersed with at least four types of acidophilic and basophilic gland cells. The epidermis rests on a well-organized nervous layer composed of nerve cells and circular and longitudinal nerve fibers. This nervous layer is condensed and thickened along the oral side of the podial column to form a prominent longitudinal strand, the podial nerve. There is a similarly thickened circular strand around the terminal sucker-disc.

Two layers of connective tissue lie below the nervous layer. The outer one is diffuse and consists of longitudinally orientated bundles of collagenous fibers interspersed with cellular elements including rounded or amoeboid cells, with small compact nuclei. The staining properties and appearance of these cells identify them as wandering coelomocytes of the type common in the coelomic cavity of the tube-foot. The collagenous elements stain deeply with the classic Ponceau S method for collagen and to varying degrees with PAS, acid fuchsin, and eosin. Occasional elastin fibers also occur, staining with aldehyde-fuchsin.

The inner layer of connective tissue is narrower than the outer one, is extremely compact, and consists entirely of circularly orientated collagenous fibers. It encircles the muscular layer, which forms the longitudinal retractor muscle, and this in turn is limited by a thin cellular epithelium lining the tube-foot lumen.

The cyst wall (Fig. 1B) is formed from starfish tissue without any contribution from the turbellarian and is essentially an extension of the tube-foot cuticle, epidermis, nervous tissue, and the outermost fibers of the longitudinal connective tissue. The turbellarian is, in effect, inserted within the outer connective tissue layer. The components of the cyst wall are continuous with their counterparts in the tube-foot. Sections of small cysts containing sexually immature turbellarians show that the cyst begins as a small blister-like swelling that increases in size as the tube-foot's epidermal, nervous, and outer connective tissues undergo localized growth to accommodate the growing parasite. As the latter increases in length, differential growth of the cyst wall produces the typical spindle- or pear-shaped cyst.

The orientation of Acholades asteris *relative to the tube-foot*

Transverse sections of infected tube-feet show that cysts containing *A. asteris* never form either under or immediately adjacent to the thickened longitudinal strand of nervous tissue. When only a single cyst is present it is almost always diametrically opposite the strand. When two or three cysts occur the larger and older one, as judged from the condition of the turbellarian's gonads (*A. asteris* is protandrous), is usually opposite the strand with the smaller cyst or cysts nearby but quite separate.

Within the cyst, the turbellarian is always orientated with what is interpreted as its morphologically anterior end directed towards the bases of the cyst and tube-foot. The gonads and associated structures, recognizable as primordia in even the smallest specimens, are always found at the other end of the body in the part lying in the distal portion of the cyst (Fig. 1A); the reproductive system is typically posterior in the Turbellaria. It was not possible to confirm this interpretation by examining the nervous system, however, as only occasional nerve cells could be identified with any certainty. These occur laterally, perhaps as components of lateral cords, but nothing resembling cerebral ganglia could be found.

The nutrition of Acholades asteris

A. asteris lacks any trace of the typical turbellarian alimentary system and feeds via its epidermis on the collagenous and cellular components of the outer connective tissue layer of the tube-foot wall.

The epidermis and subepidermal glands. The body wall consists of a monolayered epidermis, resting on a delicate basement membrane, and a thin subepidermal musculature made up of outer circular and inner longitudinal muscles. The epidermis (Figs. 2A–D) has the same basic structure over the entire body surface but the relative numbers of cell types, their sizes and contents, and the occurrence and sizes of epidermal invaginations vary in different areas. The most profound modifications occur anteriorly where the cyst is attached to the tube-foot. Over most of the body surface, posterior to this region, the epidermis is 25–30 μm in height and composed

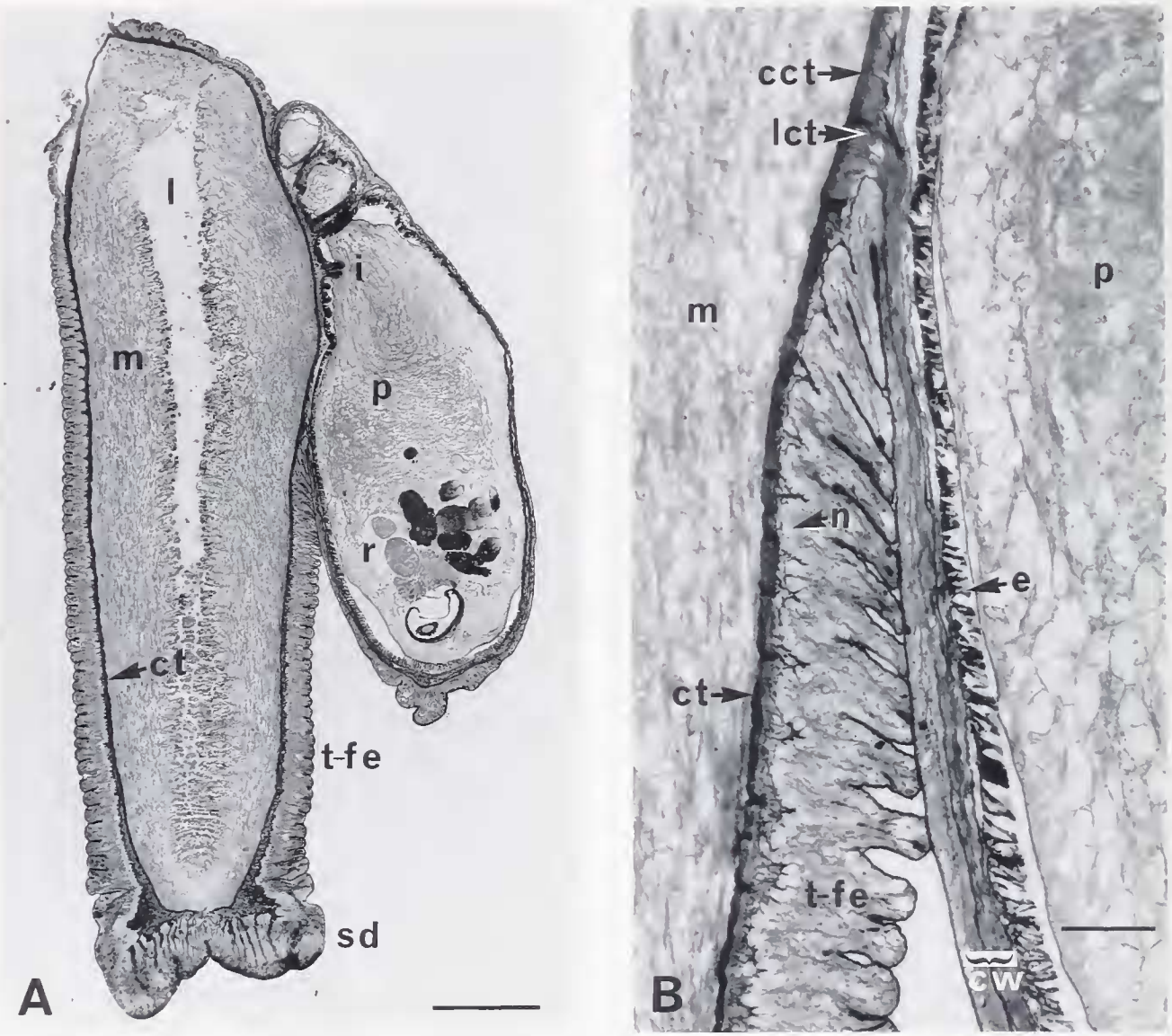

Figure 1. Longitudinal sections through a retracted ambulacral tube-foot of *Coscinasterias calamaria* with an attached cyst (right) containing a mature *Acholades asteris*. (A) An entire section showing the general anatomy of the tube-foot, cyst, and worm (section stained with alcian blue, periodic acid-Schiff and orange G; scale = 0.5 mm). (B) Detail from another section showing the structure and relationship of the tube-foot and cyst walls (stain as in (A); scale = 75 μm). cct, circular connective tissue; ct, connective tissue layers (circular and longitudinal) of tube-foot wall; cw, cyst wall; e, epidermis of *A. asteris*; i, major invaginations of *A. asteris* epidermis; l, lumen of tube-foot, lined by coelomic epithelium; lct, longitudinal connective tissue; m, muscular layer of tube-foot; n, nervous layer; p, inner compact parenchyma; r, reproductive organs of *A. asteris*; sd, sucker disc of tube-foot; t-fe, tube-foot epidermis.

mainly of ciliated columnar cells, 10–12 μm wide, with basal nuclei and various cytoplasmic inclusions similar to those to be described in the cells of the attachment area. Short microvilli occur sparsely between the cilia. Oval gland cells, 12–15 μm by 8–10 μm, occur between the basal regions of the columnar cells in a ratio of approximately 1:20 of the latter. The gland cells have basophilic inclusions, 1–3 μm in diameter, which give thermolabile positive reactions for endopeptidase when incubated in the indoxyl acetate medium for non-specific esterases at pH 4.5 in the presence of 10^{-3} *M* cysteine.

Small v-shaped invaginations, densely lined with cilia, occur along the epidermis at intervals of 30–60 μm; these may be as little as 10–15 μm deep and involve only 2–5

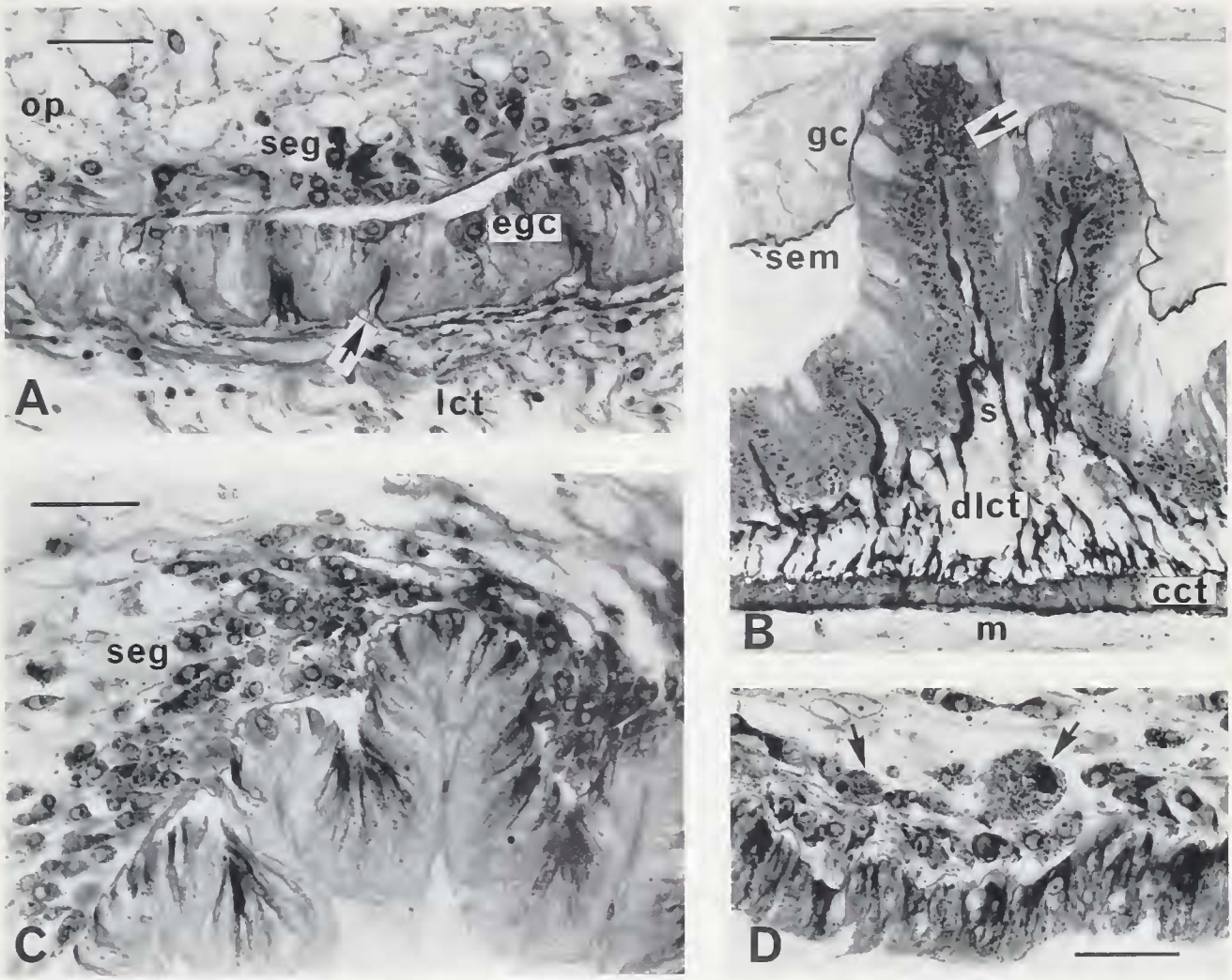

Figure 2. The epidermis and associated structures in *Acholades asteris*. (A) A portion of the epidermis in the mid-body region showing the characteristic v-shaped intercellular invaginations (arrowed) (haematoxylin and eosin; scale = 30 μm). (B) A portion of invaginated epidermis in the attachment area, showing deeply staining phagosomes (arrowed) in the distal regions of the columnar cells (alcian blue, periodic acid-Schiff and orange G; scale = 35 μm). (C) As (B) but stained to show the concentrations of subepidermal glands below the major invaginations (Giemsa; scale = 40 μm). (D) A portion of the body wall of *A. asteris* adjacent to the attachment area, showing two gametocysts of the gregarine *Monocystella* sp. (arrowed) (Giemsa; scale = 25 μm). cct, circular connective tissue of tube-foot wall; dlct, longitudinal connective tissue being digested by enzymes from *A. asteris*; egc, epidermal gland cell; gc, epidermal and subepidermal gland cells (not stained); lct, longitudinal connective tissue of cyst wall; m, muscular layer of tube-foot wall; op, outer parenchyma; s, strands of connective tissue drawn into epidermal invaginations; seg, subepidermal gland cells; sem, subepidermal musculature (contracted away from epidermis during fixation).

cells if they are at 90° to the basement membrane. When they run obliquely into the epidermis they can be more extensive and involve 5–10 cells (Fig. 2A).

Subepidermal gland cells occur at intervals in the parenchyma adjacent to the subepidermal musculature (Figs. 2A–D). The cell bodies are 30–40 μm long, basically spindle-shaped but often tapering sharply from a central expanded area containing the nucleus. They have strongly basophilic cytoplasm, with granular inclusions, and are produced at one end into long, extremely slender necks that pass outwards through the musculature and either through or between the epidermal cells to open between the bases of the cilia. The majority lie parallel to the epidermal surface so that their necks, or ducts, bend sharply as they pass outwards. The granular secretions are similar to those of the epidermal cells, being baso-

philic and showing strong reactions for acidic endopeptidase.

The epidermis becomes thicker anteriorly, with the height of the columnar cells rising to 40–60 μm. The number of gland cells also increases and their ratio to the columnar cells may rise to 1:4 of the latter (Fig. 2B). This thickened, highly glandular epidermis is deeply invaginated in the areas where it faces the split outer layer of the tube-foot's connective tissue, in the region where the cyst is attached to the tube-foot column (Figs. 1A, 2B, C). The invaginated epidermis is accompanied by the subepidermal musculature and glands; there are usually four or five major invaginations, one or two of which may extend into the parenchyma for up to a quarter of the diameter of the turbellarian's body. Their epidermal linings very often possess secondary intuckings of the type seen elsewhere over the body surface, but, like these, they involve only epidermal cells and not the underlying muscles. The columnar cells of both types of invagination remain fully ciliated.

The number of subepidermal glands increases markedly in this anterior region and are especially concentrated around the deep epidermal invaginations where they form a compact layer several cells thick (Fig. 2C). However, each gland cell retains its own duct which carries its secretions into the ciliated lumen of the invagination. The majority of the ducts pass through the columnar epidermal cells and any one of these may be traversed by several ducts; the secretion appears to be in membrane-bound globules 100–150 nm in diameter, so far as could be judged from the poorly fixed available material.

Digestion and uptake of host tissues. Endopeptic secretions from the epidermal and subepidermal glands attack the outer connective tissue layer of the tube-foot in the attachment region of the cyst, where it is exposed by the separation from it of its own outermost fibers and the tube-foot epidermis to form the cyst wall (Fig. 2B). Bundles of collagen fibers, connective tissue cells, and coelomocytes become progressively disorganized and are eventually reduced to an amorphous broth whose constituents, both particulate and fluid, show a blend of the staining reactions given by the unaltered tissue elsewhere in the tube-foot wall. This semi-digested broth occurs in large amounts in the attachment region and within the major epidermal invaginations facing the area attacked. It also bathes the turbellarian's entire epidermal surface and enters the various minor invaginations. Away from the attachment region, the turbellarian's epidermis is closely applied to the cyst wall so that only relatively small amounts are present. The inner face of the cyst wall (the extended outer connective tissue layer of the tube-foot wall) shows no evidence of digestive attack. Therefore, I conclude that the endopeptic secretions from the epidermal and subepidermal glands discharged away from the attachment region merely continue digestion of the broth. How this is achieved without damaging the cyst wall remains unknown.

The major epidermal invaginations often contain in their lumina strands of collagenous material in addition to the broth (Fig. 2B). These must have been drawn in by ciliary action, appropriate localized activity of the invaginated subepidermal musculature, or a combination of these.

Disruption of the longitudinal connective tissue usually extends inwards for about one half of its width. The layer is not breached, and the inner circular connective tissue, podial musculature, and coelomic epithelium are not affected. This situation was found in all 23 specimens examined, indicating that regeneration of the damaged layer keeps pace with the digestive attack. In many specimens the layer reacted by proliferating and thickening and the attachment area contained larger numbers of coelomocytes than were present in comparable areas of uninfected tube-feet.

The broth of digesting material is taken into the turbellarian's epidermal cells in pinocytotic vesicles which form between the bases of the cilia (Fig. 3A). The vesicles often contain whorled membranes and other particles, as well as more homogeneous materials; they move down into the cells and several fuse to form large phagosomes (Figs. 3A, B). The pinocytotic vesicles are just visible with the light microscope as tiny spheres staining with PAS and acid fuchsin. However, the phagosomes are prominent features of the epidermal cells, reacting strongly to PAS and acid fuchsin and lying in the distal to middle thirds of the cells (Fig. 2B). They are 1–3 μm in diameter when newly formed but decrease in size as they pass further into the cells and as digestion of their contents proceeds. Their shrinking is accompanied by progressive decrease in their response to PAS. In the basal regions of the cells their remnants become PAS-negative and stain only weakly with acid fuchsin or haematoxylin.

The same sequence of events occurs elsewhere in the epidermis, away from the attachment area, but to a much lesser extent. Phagosomes can usually be seen in the epidermis covering the middle region of the body, but within groups of cells rather than continuously. They are very infrequent posteriorly in the genital region.

Food reserves. The parenchyma filling the interior of the turbellarian's body is a syncytial tissue made up of an outer diffuse layer surrounding a compact and more heavily staining core (Fig. 1A). Glycogen is the principal food reserve. Large quantities seen as PAS-positive, amylase-labile granules occur in the inner parenchyma and

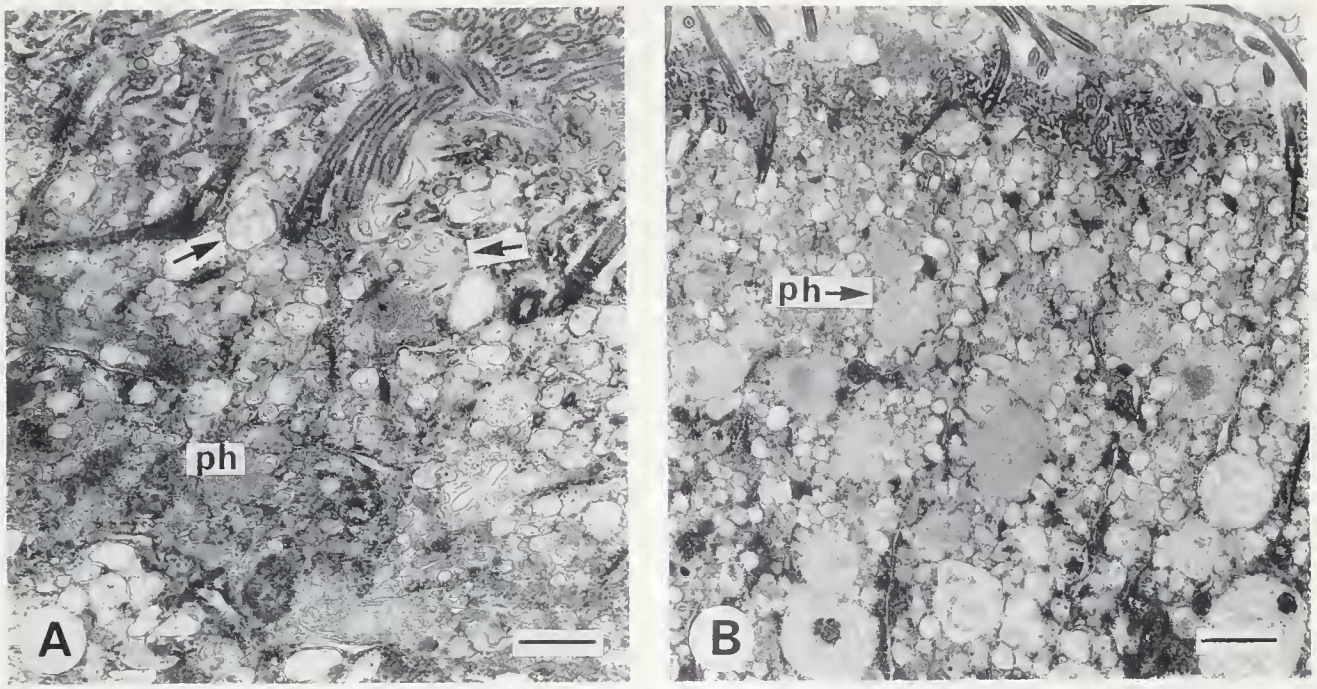

Figure 3. Transmission electron micrographs of the distal portions of epidermal cells in *Acholades asteris*. (A) Pinocytotic vesicles (arrowed) forming between the bases of the cilia (scale = 0.5 μm). (B) Pinocytotic vesicles and phagosomes formed by fusion of such vesicles packing the distal regions of five columnar epidermal cells (scale = 1 μm). ph, phagosome.

smaller amounts in the ovaries and vitellaria. Only small amounts of lipids are laid down. These occur sparingly in the inner parenchyma and in somewhat larger quantities in the ovaries and vitellaria, as osmiophilic globules 1–2 μm in diameter.

Hyperparasitic protozoa

Six of the 23 *Acholades asteris* examined were infected with an aseptate gregarine protozoan identified as an undescribed species of *Monocystella*. The gregarines occur in the anterior region of the turbellarian, in the outer parenchyma and epidermis, and are associated particularly with the large epidermal invaginations. Young trophozoites from the parenchyma invade the epidermis where they grow to a length of 40–50 μm. When fully grown they often show a more lightly staining region anterior to the nucleus, especially with Giemsa or Azur II. They apparently migrate back to the outer parenchyma, as mature gamonts, since syzygy was only seen here and never in the epidermis. Syzygy is followed by formation of isogamous gametocysts that often contain residual cytoplasm (Fig. 2D). The remainder of the life cycle is unknown. Sporocysts, oocysts, and sporozoites were not found and no stages were seen outside the turbellarian in the attachment area, cyst wall, or tube-foot.

Discussion

The principal feature of interest emerging from these observations is the unequivocal demonstration that in this unusual turbellarian, *Acholades asteris*, the epidermis and subepidermal structures function as a complete alimentary system through which the animal obtains all its food. Supplementary uptake of organic nutrients via the epidermis occurs in a wide range of free-living aquatic invertebrates (see reviews by Stephens, 1972; Jørgensen, 1976; Stewart, 1979) and in entoparasites such as some digenetic trematodes (Smyth and Halton, 1983). However, in these instances the substances taken in are already dissolved in the surrounding medium. This is also the case in fecampiid turbellarians living in hemocoels, and in cestodes. Admittedly, in the latter there is evidence that phosphatases and nonspecific esterases on the outer surfaces of the apical plasma membrane convert non-transportable organic phosphate esters into transportable organic bases and phosphate (Lumsden and Specian, 1980; Threadgold, 1984) but this is not the same as the situation found in *A. asteris*. Here, the flatworm secretes digestive enzymes from modified epidermal and subepidermal glands which attack organized components of the surrounding host tissue, degrading them into particulate or soluble substances. These are

taken into the epidermal cells by pinocytosis; digestion is completed intracellularly. The entire process is directly comparable to that occurring in the gut of most other turbellarians, whether free-living or symbiotic, after ingestion of prey organisms or host tissues by muscular pharyngeal feeding mechanisms (Jennings, 1977, 1985; Garcia-Corrales and Gamo, 1988). Particularly noteworthy is the occurrence in *A. asteris* of a large intracellular component in its digestive physiology, exactly as in other turbellarians, even though the digestive tissue is ectodermal epidermis and not entodermal gastrodermis.

The columnar cells in which digestion is completed remain densely ciliated despite the large number of pinocytotic vesicles constantly forming in their distal surfaces. The cilia are probably essential for circulation and mixing of the enzymes secreted from and through the epidermis, and for moving the semi-digested broth away from the attachment area and over the general body surface for uptake elsewhere. This retention of cilia on cell surfaces concerned with uptake of particulate as well as soluble materials is unusual but has been reported also for nemertean worms. In these it is gastrodermal cells that are involved and materials are taken up by phagocytosis, with large phagosomes forming directly and not from fusion of pinocytotic vesicles (Jennings, 1969).

The minor epidermal invaginations seen in *A. asteris* can be regarded, simply, as anatomical adaptations increasing the surface area available for enzyme-substrate activities and nutrient uptake, similar to the folds and villi-like structures found in most alimentary systems. This view can also be taken of the major invaginations but these are very specialized structures with hypertrophied cells and greatly increased numbers of gland cells; they are accompanied by invagination of the underlying musculature and proliferation of the associated subepidermal glands. They function, in fact, as miniature guts, drawing into their lumina strands of host tissue for digestion (Fig. 2B). If the orientation of *A. asteris* within the cyst has been interpreted correctly then these major invaginations, always sited together and in the same area, are seen to be anterior and sub-terminal. They are thus in the same relative position as the stomodaeal invaginations in the embryos of other turbellarians, which develop into the mouth and pharyngeal region, and certainly resemble these when seen in section if the proliferation of their glandular components is ignored. Therefore, they may represent an alimentary system that has not developed along orthodox lines and which is supplemented by concomitant modifications of the epidermis over the rest of the body.

Proliferation of sub-epidermal glands, but without regionalization and anatomical modifications of the epidermis, is a consistent feature of other parasitic turbellarians that lack normal alimentary systems, such as *Fecampia, Glandulodema*, and *Kronborgia* (Caullery and Mesnil, 1903; Jägersten, 1942; Shinn and Christensen, 1985). Their secretions, and those of epidermal glands, are concerned with cocoon formation and it is not known if they also play a part in nutrition.

A. asteris may use organic nutrients in addition to those obtained by its extra-corporeal digestion of host tissues. Epidermal uptake of dissolved organic materials such as amino acids is well documented for echinoderms and especially asteroids (Ferguson, 1971; Péquignat, 1972; Stephens *et al.*, 1978; Bamford, 1982). The tube-feet form an enormous surface area for this and thus *A. asteris* is well placed for carrier-mediated abstraction of any nutrients passing inwards from the exterior. Indeed, since the cyst wall is simply an extension of the two outer components of the tube-foot wall, with the turbellarian in effect taking the place of the inner components, nutrient absorption by the cyst wall epidermis could contribute significantly to the parasite's nutrition. Further potential sources of nutrients are the migratory coelomocytes visiting the tube-foot wall, whose numbers increase in the presence of *A. asteris*, and the hemal strands adjacent to the bases of the tube-feet. The coelomocytes and hemal system are both involved in nutrient translocation within the starfish body (Ferguson, 1982).

The favorable surface area-volume ratio of the cyst, comparable to that of the tube-foot, and the thinness of the cyst wall, will facilitate gaseous exchange in *A. asteris*. The flatworm has not evolved a specific respiratory pigment such as the hemoglobins found in other entosymbiotic turbellarians that live deeper within their respective hosts (Jennings and Cannon, 1987; Jennings, 1988). The emphasis on glycogen in the food reserves probably has no respiratory significance. It is a feature common to many entosymbiotes and is likely to be related to the reproductive physiology as discussed in detail elsewhere (Jennings and Calow, 1975).

It is clear, then, that the asteroid tube-foot, unlikely though it might seem at first sight, is a favorable habitat for *A. asteris*. The turbellarian is well adapted to it physiologically and also behaviorally. The unknown larval stage must show a considerable degree of site selection when it enters the tube-foot wall, settling within the outer connective tissue layer at a point well away from the podial nerve strand. Preservation of the strand and of the circular connective tissue layer and musculature, vital for the functioning of the tube-foot (Smith, 1946, 1947; Hyman, 1955), allows the tube-foot to remain alive and thus also preserves the cyst. The feeding activities are in balance with the host's regenerative capacities and do not involve the cyst wall which can continue to grow to accommodate the growing parasite. Cysts were not found

in the sucker-disc region but were described from there by Hickman and Olsen (1955). Their presence presumably restricts the adhesive function of the tube-feet but will not be lethal to these if the cyst is formed similarly to those occurring on the podial columns.

The occurrence of the gregarine *Monocystella* sp. in *A. asteris* is of particular interest in view of its location. *Monocystella* spp. are typically parasites of turbellarian alimentary systems (Levine, 1977). For example, the hyperparasitic *M. epibatis* has recently been described from the rhabdocoel *Pterastericola vivipara,* which is itself entoparasitic in the pyloric ceca of the Crown of Thorns starfish *Acanthaster planci* on the Great Barrier Reef (Cannon and Jennings, 1988). *M. epibatis* trophozoites and gamonts live in the gastrodermis and intestinal lumen of their host. The similarly hyperparasitic *Monocystella* sp. found in *A. asteris* retains this association with turbellarian digestive regions, its trophozoites and gamonts having colonized the epidermis and subepidermal parenchyma.

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Literature Cited

Bamford, D. 1982. Epithelial absorption. Pp. 317–330 in *Echinoderm Nutrition,* M. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

Bellon-Humbert, C. 1983. *Fecampia erythrocephala* Giard (Turbellaria Neorhabdocoela), a parasite of the prawn *Palaemon serratus* Pennant: the adult phase. *Aquaculture* **31:** 117–140.

Bresciani, J., and M. Køie. 1970. On the ultrastructure of the epidermis of the adult female of *Kronborgia amphipodicola* Christensen and Kanneworff, 1964 (Turbellaria: Neorhabdocoela). *Ophelia* **8:** 209–230.

Cannon, L. R. G. 1986. *Turbellaria of the World: A Guide to Families and Genera.* Queensland Museum, South Brisbane, 136 pp.

Cannon, L. R. G., and J. B. Jennings. 1988. *Monocystella epibatis* n. sp., a new aseptate gregarine hyperparasite of rhabdocoel turbellarians parasitic in the Crown of Thorns starfish, *Acanthaster planci* Linnaeus, from the Great Barrier Reef. *Arch. Protistenk* **136:** 267–272.

Caullery, M., and F. Mesnil. 1903. Recherches sur les *Fecampia* Giard, Turbellariés Rhabdocèles, parasites internes des Crustacés. *Ann. Fac. Sci. Marseille.* **13:** 1–37.

Christensen, A. M. 1981. The geographical and bathymetrical distribution of the Fecampiidae (Turbellaria, Rhabdocoela). *Hydrobiologia* **84:** 13–16.

Ferguson, J. C. 1971. Uptake and release of free amino acids by starfishes. *Biol. Bull.* **141:** 122–129.

Ferguson, J. C. 1982. Nutrient translocation. Pp. 373–393 in *Echinoderm Nutrition,* M. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

Garcia-Corrales, P., and J. Gamo. 1988. Ultrastructural changes in the gastrodermal phagocytic cells of the planarian *Dugesia gonocephala* s.l. during food digestion (Plathelminthes). *Zoomorphology* **108:** 109–117.

Hassall, M., and J. B. Jennings. 1975. Adaptive features of gut structure and digestive physiology in the terrestrial isopod *Philoscia muscorum* (Scopoli) 1763. *Biol. Bull.* **149:** 348–364.

Hickman, V. V., and A. M. Olsen. 1955. A new turbellarian parasitic in the sea-star *Coscinasterias calamaria* (Gray). *Pap. Proc. R. Soc. Tasmania* **89:** 55–63.

Holt, P. A., and D. F. Mettrick. 1975. Ultrastructural studies of the epidermis and gastrodermis of *Syndesmis franciscana* (Turbellaria: Rhabdocoela). *Can. J. Zool.* **53:** 536–549.

Holt, S. J. 1958. Studies in enzyme histochemistry. *Proc. R. Soc. Lond. B* **148:** 465–532.

Hyman, L. H. 1955. *The Invertebrates: Echinodermata, Vol. IV.* McGraw-Hill, New York, 763 pp.

Jägersten, G. 1942. Zur Kenntnis von *Glanduloderma myzostomatis* n.gen., n.sp., einer eigentümlichen, in Myzostomiden Schmarotzenden Turbellarienform. *Ark. Zool.* **33A:** 1–24.

Jennings, J. B. 1969. Ultrastructural studies on the phagocytic uptake of food materials by the ciliated cells of the rhynchocoelan intestine. *Biol. Bull.* **137:** 476–485.

Jennings, J. B. 1971. Parasitism and commensalism in the Turbellaria. *Adv. Parasitol.* **9:** 1–32.

Jennings, J. B. 1977. Patterns of nutrition in free-living and symbiotic Turbellaria and their implications for the evolution of entoparasitism in the phylum Platyhelminthes. *Acta Zool. Fenn.* **154:** 63–79.

Jennings, J. B. 1985. Feeding and digestion in the aberrant planarian *Bdellasimilis barwicki* (Turbellaria: Tricladida:Procerodidae): an ectosymbiote of freshwater turtles in Queensland and New South Wales. *Aust. J. Zool.* **33:** 317–327.

Jennings, J. B. 1988. Nutrition and respiration in symbiotic Turbellaria. *Fortschr. Zool.* **36:** 1–13.

Jennings, J. B., and P. Calow, 1975. The relationship between high fecundity and the evolution of entoparasitism. *Oecologia* (Berl.) **21:** 109–115.

Jennings, J. B., and L. R. G. Cannon. 1985. Observations on the occurrence, nutritional physiology and respiratory pigment of three species of flatworms (Rhabdocoela: Pterastericolidae) entosymbiotic in starfish from temperate and tropical waters. *Ophelia* **24:** 199–215.

Jennings, J. B., and L. R. G. Cannon. 1987. The occurrence, spectral properties and probable rôle of haemoglobins in four species of entosymbiotic turbellarians (Rhabdocoela: Umagillidae). *Ophelia* **27:** 143–154.

Jennings, J. B., and J. I. Phillips. 1978. Feeding and digestion in three entosymbiotic graffillid rhabdocoels from bivalve and gastropod molluscs. *Biol. Bull.* **155:** 542–562.

Jondelius, U. 1986. Epidermal ultrastructure of *Anoplodium stichopi* (Platyhelminthes, Dalyellioida), a flatworm endosymbiotic in *Stichopus tremulus* (Holothuroida). *Zoomorphology* **106:** 254–259.

Jørgensen, C. B. 1976. August Pütter, August Krogh and modern ideas on the use of dissolved organic matter in aquatic environments. *Biol. Rev.* **51:** 291–328.

Kent, M. L., and A. C. Olson. 1986. Interrelationships of a parasitic turbellarian, (*Paravortex* sp.) (Gralfillidae, Rhabdocoela) and its marine fish host. *Fish Pathol.* **21:** 65–72.

Levine, N. D. 1977. Revision and checklist of the species (other than

Lecudina) of the aseptate gregarine family Lecudinidae. *J. Protozool.* **24:** 41–52.

Lumsden, R. D., and R. Specian. 1981. The morphology, histology and fine structure of the adult stage of the cyclophyllidean tapeworm *Hymenolepis diminuta*. Pp. 157–280 in *Biology of the Tapeworm* Hymenolepis diminuta, H. P. Arai, ed. Academic Press, London.

MacKinnon, B. M., M. D. B. Burt, and A. W. Pike. 1981. Ultrastructure of the epidermis of adult and embryonic *Paravortex* species (Turbellaria, Eulecithophora). *Hydrobiologia* **84:** 241–252.

Pearse, A. G. E. 1972. *Histochemistry: Theoretical and Applied, 3rd ed.* Churchill Livingstone, Edinburgh. 1518 pp.

Péquignat, E. 1972. Some new data on skin digestion and absorption in urchins and sea stars (*Asterias* and *Henricia*). *Mar. Biol.* **12:** 28–41.

Shinn, G. 1981. The diet of three species of umagillid neorhabdocoel turbellarians inhabiting the intestine of echinoids. *Hydrobiologia* **84:** 155–162.

Shinn, G., and A. M. Christensen. 1985. *Kronborgia pugettensis* sp. nov. (Neorhabdocoela: Fecampiidae), an endoparasitic turbellarian infesting the shrimp *Heptacarpus kincaidi* (Rathbun) with notes on its life-history. *Parasitology* **91:** 431–437.

Smith, J. E. 1946. The mechanics and innervation of the starfish tube foot-ampulla system. *Phil. Trans. R. Soc. Ser. B.* **232:** 279–310.

Smith, J. E. 1947. The activities of the tube feet of *Asterias rubens* L. 1. The mechanics of movement and posture. *Q. J. Microsc. Sci.* **88:** 1–14.

Smyth, J. D., and D. W. Halton. 1983. *The Physiology of Trematodes, 2nd edn.* Cambridge Univ. Press, Cambridge. 446 pp.

Stephens, G. C. 1972. Amino acid accumulation and assimilation in marine organisms. Pp. 155–184 in *Nitrogen Metabolism and the Environment*, J. W. Campbell and L. Goldstein, eds. Academic Press, London.

Stephens, G. C., M. J. Volk, S. H. Wright, and P. S. Backlund. 1978. Transepidermal accumulation of naturally occurring amino acids in the sand dollar, *Dendraster excentricus*. *Biol. Bull.* **154:** 335–347.

Stewart, M. G. 1979. Absorption of dissolved organic nutrients by marine invertebrates. *Oceanogr. Mar. Biol. Ann. Rev.* **17:** 163–192.

Threadgold, L. T. 1984. Parasitic Platyhelminthes. Pp. 132–191 in *Biology of the Integument, Vol. 1, Invertebrates*, J. Bereiter-Hahn, A. G. Matolsky, and K. S. Richards, eds. Springer-Verlag, Berlin.

Tyler, S. 1984. Turbellarian Platyhelminthes. Pp. 112–131 in *Biology of the Integument, Vol. 1, Invertebrates*, J. Bereiter-Hahn, A. G. Matolsky and K. S. Richards, eds. Springer-Verlag, Berlin.